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Markers of Bone Mineral Metabolism and Cardiac Structure and Function in Perinatally HIV-Infected and HIV-Exposed but Uninfected Children and Adolescents

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Abstract

Background: Disordered bone mineral metabolism and low vitamin D concentrations are associated with cardiovascular abnormalities; few studies have evaluated this relationship in HIV-infected youth.

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Setting: Adolescent Master Protocol (AMP) is a Pediatric HIV/AIDS Cohort Study (PHACS) network study conducted across 14 United States sites.

Methods: Among perinatally HIV-infected (PHIV) and HIV-exposed uninfected (PHEU) youth enrolled in AMP, we evaluated associations of vitamin D (measured as 25-hydroxyvitamin D [25OHD]), parathyroid hormone (PTH), calcium, phosphate, and fibroblast growth factor-23 (FGF-23) concentrations with echocardiographic measures of left ventricular (LV) structure, function and concentrations of NT-proBNP, a biomarker of cardiac damage.

Results: Among 485 participants (305 PHIV, 180 PHEU) with echocardiograms and bone mineralization measures, low 25OHD (< 20 ng/mL) was common among all participants (48% PHIV and 44% PHEU), but elevated PTH (> 65 pg/mL) was identified more often among PHIV than PHEU participants (9% vs 3%, p=0.02). After adjusting for HIV status and demographic covariates, both low 25OHD and elevated PTH were associated with lower mean LV mass z-scores, while elevated PTH was associated with higher mean fractional shortening z-scores. Participants with low 25OHD also had slightly higher mean LV end-systolic wall stress z-scores, but differences were more pronounced in PHEU than in PHIV participants. FGF-23 was inversely related to end-diastolic septal thickness both overall and among PHIV participants.

Conclusion: In this cohort of PHIV and PHEU youth, we observed associations of 25OHD, PTH, and FGF-23 with both structural and functional cardiac parameters, supporting links between bone mineral metabolism and cardiac status.

Keywords

25-hydroxy-vitamin D; parathyroid hormone; cardiac function; HIV infection; children

Introduction

Disordered bone metabolism and low bone mineral density are common in both adults and children with HIV disease[1–3]. We have demonstrated lower total body and lumbar spine bone mineral density in children with perinatal HIV infection (PHIV) than in those who were perinatally HIV-exposed but uninfected (PHEU)[3], along with a high prevalence (40%) of vitamin D insufficiency (VDI) in both PHIV and PHEU children[4]. Increasing evidence suggests that VDI is associated with several common cardiovascular abnormalities. An analysis of over 41,000 adults demonstrated highly significant inverse associations of 25-hydroxyvitamin D (25OHD) levels and the prevalence of type 2 diabetes mellitus, systemic hypertension, dyslipidemias, and peripheral vascular disease[5]. In a group of HIV-infected youth, standard doses of 25OHD supplementation (18,000 IU/month vitamin D3) were shown to decrease carotid bulb intimal medial thickness, a marker for subclinical atherosclerosis[6]. Various mechanisms have been proposed to explain 25OHD contributions to the development of cardiovascular disorders, in both HIV and general populations, including inflammation, alterations in insulin sensitivity, and inhibition of the renin-angiotensin-aldosterone axis.[6,7]

Derangements in other factors affecting bone mineralization, such as parathyroid hormone (PTH) and phosphate, have also been associated with subclinical myocellular damage. In pediatric patients with chronic renal failure, higher cardiac troponin T (cTnT), a marker of

myocardial injury, was associated with reduced PTH and elevated phosphate[8]. Low PTH concentrations have also been associated with left ventricular dilation in adults with chronic renal failure, although the mechanism underlying this relationship is not clear.

While disordered mineral metabolism is associated with cardiovascular disease, establishing a causal relationship is difficult because bone mineral metabolism markers are highly linked and involved in complex regulatory feedback loops. Recently, fibroblast growth factor-23 (FGF-23) has been found to be a primary regulator of phosphate metabolism. FGF-23 is a peptide hormone secreted primarily by osteocytes. It reduces the reabsorption of phosphate in the proximal renal tubules, reducing systemic 1,25-dihydroxyvitamin D (1,25OHD) concentrations through diminished production and increased degradation of 1,25OHD and suppression of PTH transcription and secretion[9]. Elevated concentrations of FGF-23 directly correlate with increased left ventricular (LV) mass in patients with chronic kidney disease[10]. Given its importance in other clinical situations, evaluation of a potential role for FGF-23 in the cardiovascular status of children with HIV and those exposed prenatally to HIV is warranted.

Even though abnormal serum concentrations of bone mineral metabolism (BMM) markers (i.e., 25OHD, PTH, calcium, phosphate, and FGF-23) are common in HIV-infected populations, particularly among those receiving combination antiretroviral therapy[4,11,12], and are associated with adverse cardiovascular conditions including congestive heart failure[13,14], it is unknown whether these markers are associated with echocardiographic findings or with NT-proBNP, a biomarker of cardiac function. The objectives of this study were to evaluate associations of BMM marker concentrations with cardiac structure and function (as measured by echocardiography and NT-proBNP) in a population of PHIV and PHEU children, and to descriptively evaluate whether these associations vary by HIV infection status. Additionally, given the higher incidence of VDI in persons of black race, associations within this specific population were separately explored.

Methods

The National Institutes of Health-funded Adolescent Master Protocol (AMP) is a prospective cohort study conducted by the Pediatric HIV/AIDS Cohort Study (PHACS) network at 14 sites across the United States. Patients were enrolled between March 2007 and November 2009, with ongoing follow-up visits. The study was designed to define the impact of HIV infection and antiretroviral therapy on pre-adolescents and adolescents infected perinatally (PHIV). A group of perinatally HIV-exposed but uninfected children (PHEU) within the same age range and similar sociodemographic backgrounds was also enrolled. The study was approved by each site's Institutional Review Board and by the Harvard T.H. Chan School of Public Health, Boston, MA. Written informed consent was provided by each parent or guardian, and assent was obtained as directed by the Institutional Review Board at each study site.

Measures of bone metabolism

BMM markers, including 25OHD, PTH, calcium, phosphate, and FGF-23, were measured in PHIV and PHEU children in AMP who had an adequate echocardiogram performed within

one year of the sample for BMM markers. Full details of the BMM marker assays used have been reported by Jacobson et al[4]. FGF-23 was measured by ELISA (Kainos Laboratories) and levels were log10-transformed to more closely approximate a normal distribution. In most cases, duplicate aliquots were assayed and the arithmetic mean reported. To enhance clinical interpretation, 25OHD and PTH concentrations were dichotomized: those with 25OHD levels < 20 ng/mL were defined as having VDI and those with PTH values > 65 pg/mL were considered to have an elevated PTH.

Echocardiographic variables and NT-proBNP concentrations

PHACS-trained site staff obtained a single echocardiogram from each patient, with all measurements performed centrally at the echocardiographic core laboratory at Children's Hospital Boston to enhance reproducibility[15]. Z-scores were calculated based on a reference population of healthy children. Echocardiographic z-scores for the following LV function parameters were obtained: contractility, LV ejection fraction, and fractional shortening. Cardiac structure was assessed with echocardiographic z-scores for LV mass, LV end-diastolic (ED) volume, LV end-systolic (ES) volume, LV ED wall thickness, LV ED dimension, M-mode thickness-to-dimension ratio, ES wall stress, and ED septal thickness. Concentrations of NT-proBNP were measured by standard techniques at the University of Miami (Dr. Armando Mendez). Values below the detection limit were reported as less than 5 pg/mL. Concentrations of NT-proBNP were log10-transformed to achieve a more normal distribution. The cardiac measures of primary interest were fractional shortening, LV mass, contractility, LV ED wall and septal thicknesses, and NT-proBNP concentrations. Indicators of worse cardiac status included: lower z-scores for fractional shortening and contractility higher z-scores for LV mass, LV ED wall thickness, and septal thickness; and higher levels of NT-proBNP.

Potential confounders

The following variables were considered as potential confounders or independent predictors of the echocardiographic and NT-proBNP outcomes: age, race, ethnicity, sex, body mass index (BMI), region (Northeast, Midwest, South, West, or Puerto Rico), and physical activity (hours per day based on the BLOCK physical activity assessment). For analytic purposes, physical activity was categorized into four groups (< 30, 30–60, 60–120, and >120 min/day), and was considered as an ordinal variable after confirming trends in outcomes. While seasonality is well known to affect vitamin D levels, it would not be expected to have an effect on the outcome measures (echocardiographic variables and NT-proBNP concentrations). Preliminary analysis confirmed that seasonality did not affect outcomes and also had little impact on estimated associations of bone metabolism markers with the outcomes, and therefore is not included here as a potential confounder.

Among PHIV youth, the distribution of HIV disease severity measures (*i.e.*, CD4, HIV-1 RNA viral load, and CDC class) and type and duration of antiretroviral treatment (ART) were considered. CD4 and HIV measures were included in the analysis if they were obtained between 6 months prior to or within 7 days after the date of the echocardiogram. Duration of ART regimens was calculated up to the date of the echocardiogram.

Statistical Methods

Comparisons of mean echocardiographic measurements by HIV status, VDI, and elevated PTH status were conducted with two-sample T-tests and Wilcoxon rank sum tests for continuous variables and with Fisher's exact test or Pearson Chi-Square test for categorical measures. A similar approach was used to compare median BMM marker concentrations by HIV status.

Univariable and multivariable linear regression models were fit to evaluate the association of BMM marker concentrations with cardiac outcomes of interest. 25OHD and PTH were analyzed as dichotomous predictors as previously noted, while all other BMM markers (calcium, phosphate, and $\log_{10}$ FGF23) were analyzed as continuous predictors. In addition to considering low 25OHD and elevated PTH concentrations separately, we also considered a combined measure of either low 25OHD and/or elevated PTH concentrations. However, the results of the combined measures were largely similar to those for low 25OHD and are not reported here. For the cardiac biomarker outcome, $\log_{10}$ NT-proBNP, sensitivity analyses were conducted using censored linear regression models to take into account values that were left-censored because they were below the detection limit.

Multivariable linear models for most cardiac outcomes of interest were adjusted by HIV status, age at echocardiogram, sex, race, BMI z-score, and physical activity. However, for fractional shortening, adjusted models included HIV status, region, and physical activity, while those evaluating the association of ES wall stress with BMM marker concentrations were adjusted by HIV status, age, region, and physical activity. These confounders were selected based on *a priori* decisions based on the literature, and by observing the change in effect estimates when adding each covariate separately to our regression models.

We evaluated whether the association of 25OHD status with echocardiographic variables and $\log_{10}$ NT-proBNP concentrations varied by HIV infection status both descriptively, by calculating adjusted least square means for echocardiographic z-scores by 25OHD status within each HIV infection group (calculated at mean levels of all other covariates in model), and with interaction terms between HIV status and VDI in adjusted linear regression models. Exploratory analyses were also conducted to assess the association of BMM marker concentrations with cardiac outcomes among the cohort of patients with self-reported black race, who are more likely to have VDI.

SAS version 9.4 was used for all statistical analyses. All statistical tests were two-sided with p-value < 0.05 indicating significance, although emphasis was placed on consistency of results across analyses.

Results

Of the 678 enrolled participants, 513 had evaluable echocardiograms and no history of congenital cardiac disorders. Among these 513 participants, 485 youth (95%; 305 PHIV and 180 PHEU) had all or some BMM marker measurements obtained, with most on the same date as the echocardiogram. A smaller subgroup of 404 youth had NT-proBNP

measurements and, of these, 383 (95%; 231 PHIV and 152 PHEU) had at least one BMM marker measurement available (Supplemental Figure 1).

Table 1 presents the baseline sociodemographic characteristics by cohort of the 485 youth with evaluable echocardiograms and BMM markers. PHIV youth were older (mean age 12.9 years) at the time of the echocardiogram and BMM marker measurements, and more often black (76%) and non-Hispanic (76%) than PHEU youth (mean age 11.1 years, 66% black, and 63% non-Hispanic). Systemic hypertension was rare and only observed among the PHIV youth (3%). For the subset of youth with available NT-proBNP and BMM markers, sociodemographic characteristics were similar to those shown in Table 1.

Among the PHIV youth in our study population, the majority were relatively healthy at the time of their echocardiogram, with most having high CD4 counts (>350 cells/mm^3) and 68% having suppressed viral load. However, 25% had a prior AIDS-defining diagnosis (*e.g.*, CDC Class C classification) and a high percentage of PHIV youth had a lifetime peak viral load exceeding 100,000 copies/mL (Table 1).

Summary of BMM marker measurements and echocardiographic variables

The 485 participants with evaluable echocardiograms and 25OHD specimens had a high prevalence of VDI, which was similar in the two groups (48% PHIV and 44% PHEU) (Table 2). Children with VDI were significantly older than those with normal 25OHD concentrations (12.7 vs 11.8 years, respectively), more often black and Non-Hispanic, and had lower daily intake of Vitamin D (data not shown). Overall, the prevalence of elevated PTH was low (7%), but was significantly higher among PHIV (9%) than PHEU (3%) youth. PHIV youth had significantly lower median calcium and phosphate levels than PHEU, and tended to have higher FGF-23 concentrations than PHEU youth (Table 2).

PHIV youth showed significant differences in echocardiographic measures of LV structure and function compared to PHEU youth. In particular, PHIV youth demonstrated marginally lower LV ejection fraction and significantly lower fractional shortening than PHEU youth, and also had higher LV mass, LV ED and ES volumes, and LV ED dimension than PHEU youth. No difference was noted between groups in terms of log10 NT-proBNP (mean=1.51 vs 1.54 pg/mL, respectively). These findings are consistent with previously published reports from this cohort (13).

Associations of BMM markers with echocardiographic measures and NT-proBNP concentrations

Cohort-wide, in unadjusted models, participants with VDI had statistically significantly higher mean systemic blood pressure Z-scores (−0.34 vs −0.57, p=0.006). However, they had lower mean Z-scores for LV mass, LV ED volume, and LV ES volume; youth with VDI also had significantly lower mean NT-proBNP concentrations than those with normal 25OHD (Table 3). Echocardiographic z-scores rarely differed significantly by PTH status, although participants with elevated PTH had a significantly higher mean LV ED wall thickness in the unadjusted analysis (Table 4). Unadjusted models of echocardiographic outcomes indicated a significant positive association of ES wall stress with increasing

calcium levels, as well as a positive association between phosphate levels and LV ED wall thickness (data not shown).

After adjusting for HIV status and demographic characteristics, the above findings were attenuated. Adjusted mean z-scores generally no longer differed significantly by 25OHD (see Table 3) or PTH (Table 4) status. However, the association of VDI with significantly lower LV mass persisted. In addition, participants with elevated PTH concentrations had significantly higher fractional shortening and lower LV mass. In adjusted models, the positive association of calcium with ES wall stress persisted (beta=0.25, p=0.01) and a negative association was observed between calcium and $\log_{10}$ NT-proBNP (beta=−0.16, p=0.001). FGF-23 demonstrated a significant negative association with ED septal thickness (beta=−0.67, p=0.02).

Variations in associations by HIV infection status and within the PHIV group

Associations of 25OHD with echocardiographic parameters and $\log_{10}$ NT-proBNP were evaluated to determine whether they varied by HIV infection status when adjusted for the covariates described. The association of VDI with lower mean z-scores for LV mass was similar within both PHIV and PHEU youth (data not shown). However, the association of VDI with ES wall stress was modified by HIV status, with similar means among PHIV youth with low and normal 25OHD (adjusted mean z-scores, −1.11 and −1.14, respectively), but higher ES wall stress among PHEU youth with low as compared to normal 25OHD (adjusted mean z-scores, −1.04 vs −1.51, interaction p=0.05). Within the PHIV group, neither VDI nor elevated PTH was significantly associated with any echocardiographic variable or with NT-proBNP concentrations in adjusted models. However, as in the overall sample, FGF-23 concentrations in the PHIV group were negatively associated with ED septal thickness (p=0.02) and higher calcium concentrations were associated with lower NT-proBNP (p<0.001).

Associations between echocardiogram z-scores and BMM markers within racial subgroups

Analyses were conducted to determine whether the associations of BMM marker concentrations with cardiac outcomes were more pronounced among black participants. Many of the previously noted findings maintained the direction of association as the group-wide analyses above, but were no longer statistically significant. The findings that demonstrated statistical significance are presented in Table 5. A significant association between 25OHD and contractility was observed in unadjusted analysis that was not present in the overall population, but none of the 25OHD associations remained significant after adjustment. Higher calcium was associated with higher LV ED dimension and lower NT-proBNP, even after adjustment. The association between calcium and LV ED dimension was stronger among black youth than in the overall population. After adjustment, both calcium and phosphate concentrations were associated with lower NT-proBNP. Negative associations between FGF-23 and both ED septal thickness and M-mode thickness-to-dimension ratios were also noted after adjustment. There were insufficient numbers within other racial subgroups to justify such exploratory analyses.

Discussion

Abnormalities in BMM markers, especially low 25OHD concentrations, have long been associated with adverse cardiovascular conditions such as systemic hypertension, peripheral vascular disease, coronary artery disease, and congestive heart failure (CHF)[5]. The prevalence of other cardiovascular risk factors associated with these conditions, including hypercholesterolemia, insulin resistance, and lipodystrophy, are increased in children with HIV, particularly those taking protease inhibitors and nucleoside reverse transcriptase inhibitors[16–18]. Earlier detection of risk factors for development of these conditions would allow more timely and perhaps more effective interventions to prevent end-organ damage.

In this study of PHIV and PHEU children and adolescents, we sought to determine whether circulating concentrations of various BMM markers were associated with echocardiographic and cardiac biomarker abnormalities and, therefore, could potentially be early markers of altered cardiovascular health. We also evaluated whether any identified associations might be affected by HIV infection status (PHIV vs. PHEU). After adjustment for confounders, we found that participants with VDI or with elevated PTH had significantly lower LV mass z-scores than those with normal concentrations of these two BMM markers. We also found that PTH levels were positively related to fractional shortening z-scores. In comparing the PHIV and PHEU groups, the only association modified by HIV status was the association between VDI and ES wall stress z-score: the difference in z-score values between those with normal 25OHD levels and those with VDI was larger in PHEU than in PHIV. When analysis was restricted to only the PHIV population, $\log_{10}$ FGF-23 was inversely related to ED septal thickness z-scores.

The associations of BMM markers with cardiac outcomes of LV wall thickness and mass in this study were not as originally hypothesized. VDI, elevated PTH levels, and elevated FGF-23 levels have all been associated with LV hypertrophy in various populations including: 25OHD in essential hypertension[19] and chronic kidney disease[20]; PTH in coronary artery disease[21] and primary hyperparathyroidism[22]; and FGF-23 in chronic kidney disease[23]. However, the significant findings reported here were in the opposite directions: VDI was associated with lower LV mass, as was elevated PTH. Thus, VDI and elevated PTH concentrations were associated with LV mass z-scores that were closer to the population norm. Additionally, ED septal thickness z-scores were inversely related to $\log_{10}$ FGF-23 concentrations among all participants and also within the PHIV group. It is important to note that the mean z-scores for the cardiac outcome measures were within the normal range (between −2 and +2) for both functional and structural measures. The incidence of dilated cardiomyopathy in HIV disease has decreased dramatically in association with the advent of widespread ART utilization[24]. In the Pediatric Pulmonary and Cardiovascular Complications of HIV Study cohort, which enrolled subjects between May 1990 through April 1993, and collected serial echocardiographic data from birth through 10 years of age, 44% had cardiomyopathy, while only 4% of the AMP HIV-infected children, enrolled between 2007 and 2009 and aged 7–16 years, did[15]. Because they are now uncommon, the ability to identify associations with more extreme cardiac abnormalities is unlikely. In the current relatively healthy population, other mechanisms or unknown additional confounders may be present, resulting in the unexpected observed associations.

Few significant associations between BMM markers and cardiac functional measures (such as ejection fraction) were identified, despite the recognized association of derangements in mineral metabolism with clinical CHF. After adjusting for confounders, only the positive association of PTH on fractional shortening remained significant, an association that has been demonstrated in pediatric chronic renal failure where lower PTH levels have been shown to be associated with increases in CTnT (a biomarker of cardiac injury)[8] and reduced cardiac function[25].

Although the incidence of VDI and high PTH was higher in black youth[4], their associations with cardiac outcomes were not statistically significant after adjusting for confounders. However, some associations between other components (calcium, phosphate, and FGF-23) and cardiac outcomes remained significant. For example, higher calcium levels and lower phosphate levels were associated with lower NT-proBNP levels, and higher FGF-23 levels were associated with lower thickness-to-dimension ratios and ED septal thickness (therefore, a tendency toward less hypertrophy). The incidence of type 2 diabetes, CHF, and stroke is higher in black persons[26–28], and it is important to note that, although data are limited, cardiovascular risk factors, including insulin resistance, are highly prevalent in lower resource settings, as evaluated in the Children with HIV in Africa-Pharmacokinetics and Adherence/Acceptability of Simple Antiretroviral Regimens (CHAPAS)-3 trial.[29] Cardiometabolic abnormalities such as dyslipidemia and insulin resistance are also highly prevalent in other lower resourced pediatric HIV populations, as detailed by Padmapriyadarsini and colleagues[30] Identifying screening tests to allow early interventions to prevent these conditions may be of particular benefit in these groups.

Strengths and Limitations of the Study

The PHACS AMP study cohort is comprised of large, well-characterized groups of PHIV and PHEU youth with echocardiograms performed by protocol and analyzed by a core laboratory and BMM marker samples obtained in conjunction with the echocardiogram. Only 33 (7%) of the 485 participants had elevated PTH concentrations; thus, the statistical power for comparisons in this group was limited. Originally, the study also sought to evaluate associations within the PHIV group by disease severity and type of antiretroviral medication, but the lack of observed associations made such evaluations unnecessary. Since only a single echocardiogram was conducted in each participant and BMM markers were measured only once within 6 months of the echocardiogram, we do not know the temporal relationship between our exposures and outcomes. Thus, we can only address association and not causation.

Conclusion

After adjusting for HIV status and demographic covariates, those with VDI had lower mean z-scores for LV mass, whereas those with elevated PTH had a higher mean z-score for fractional shortening and a lower mean z-score for LV mass. When analysis was restricted to only PHIV, few significant findings remained following adjustment for confounders, but $\log_{10}$ FGF-23 was inversely related to end-diastolic septal thickness. Associations were, therefore, noted in both structural and functional cardiac parameters, suggesting possible

links between bone mineral metabolism and cardiac status. By establishing expected relationships between commonly assessed parameters of cardiovascular health, such as structural and functional measures by echocardiography and cardiovascular biomarkers such as NT-proBNP, future interventional efforts with 25OHD supplementation may be facilitated. Furthermore, longitudinal studies may provide an opportunity to further clarify whether additional mechanisms or unmeasured confounders are responsible for the changes seen in structural and functional cardiac parameters. Finally, from a clinical standpoint, evidence of a link between bone mineral metabolism and cardiovascular outcomes provides clinicians with another potential mechanism of cardioprotection in this vulnerable population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Sociodemographic and health characteristics among 485 youth from the Adolescent Master Protocol of the Pediatric HIV/AIDS Cohort Study with echocardiograms and bone mineralization metabolism markers

Sociodemographic Characteristics[1]	Cohort	
	Perinatally HIV-Infected (PHIV) (N=305)	Perinatally HIV-exposed Uninfected (PHEU) (N=180)
Age, Mean (SD), years	12.89 (2.75)	11.09 (2.52)
Gender, n (%)		
Male	141 (46%)	88 (49%)
Female	164 (54%)	92 (51%)
Race, n (%)		
White/Other	69 (24%)	59 (34%)
Black	220 (76%)	117 (66%)
Hispanic Ethnicity	72 (24%)	65 (37%)
Body mass index z-score (Mean [SD]), z-score	0.40 (1.21)	0.70 (1.30)
Region, n (%)		
Northeast	112 (37%)	53 (29%)
Midwest	50 (16%)	16 (9%)
South	99 (33%)	58 (32%)
West	31 (10%)	30 (17%)
Puerto Rico	13 (4%)	23 (13%)
Dietary measures, Median (IQR)		
Total daily dietary intake of Vitamin D (IU)	172 (100, 345)	134 (82, 238)
Total daily dietary intake of Calcium (mg)	727 (508, 1,005)	683 (502, 1000)
Percent of calories from fat	34.0 (30.9, 37.9)	34.7 (31.0, 38.0)
Percent of calories from protein	13.8 (12.3, 15.3)	14.0 (12.4, 15.8)
Calories (0.001 kcal)	1.79 (1.20, 2.44)	1.53 (1.11, 2.14)
Moderate-to-vigorous physical activity		
< 30 min/day	74 (28%)	38 (23%)
30–60 min/day	51 (20%)	33 (20%)
60–120 min/day	61 (23%)	51 (30%)
≥ 120 min/day	77 (29%)	46 (27%)
Systemic hypertension, n (%)	8 (3%)	0 (0%)
CD4 T-cell count (cells/μL) < 350 cells/μL, n (%)	32 (11%)	
Nadir CD4 T-cell percent < 15%, n (%)	104 (35%)	
HIV-1 RNA (copies/mL) ≤ 400 copies/mL, n (%)	203 (68%)	
Peak HIV-1 RNA (copies/mL) ≥ 100,000 copies/mL, n (%)	224 (75%)	
ART regimen at assessment, n (%)		
ART with protease inhibitor	211 (69%)	
ART without protease inhibitor	57 (19%)	
Non-ART ARV	19 (6%)	
Not on ARV	17 (6%)	
Duration on cART, Median (IQR), years	8.92 (6.27, 10.59)	

Sociodemographic Characteristics[1]	Cohort	
	Perinatally HIV-Infected (PHIV) (N=305)	Perinatally HIV-exposed Uninfected (PHEU) (N=180)
CDC HIV Class C, n (%)	76 (25%)	

SD=standard deviation, IQR=interquartile range (25^{th}, 75^{th} percentiles), PI=protease inhibitor

[1]Some characteristics were not measured or obtained for certain variables, including race (n=20; 16 PHIV 4 PHEU), ethnicity (n=3, all PHEU), dietary measures (n=28; 17 PHIV, 11 PHEU), physical activity from BLOCK physical activity questionnaire; not completed (n=45; 42 PHIV, 12 PHEU). CD4 and HIV-1 RNA measures were included if obtained 183 days prior to and up to 7 days after the date of the echocardiogram.

Table 2.

Distribution of concentrations of bone mineralization metabolism markers by HIV status among 485 youth from the Adolescent Master Protocol of the Pediatric HIV/AIDS Cohort Study with evaluable echocardiograms and serum specimens

	Cohort		
	Perinatally HIV-Infected (PHIV) (N=305)	**Perinatally HIV-exposed Uninfected (PHEU) (N=180)**	**P-Value[2]**
Serum concentrations, median (IQR)[1]			
25OHD (ng/mL)	20.3 (15.3, 25.9)	20.9 (17.0, 26.7)	0.32
Calcium (mg/dL)	9.52 (9.22, 9.80)	9.65 (9.39, 9.92)	< 0.001
Phosphate (mg/dL)	4.48 (4.01, 4.88)	4.82 (4.36, 5.21)	< 0.001
PTH (pg/mL)	32.4 (23.5, 45.0)	28.6 (22.0, 40.0)	0.005
FGF-23 (RU/mL)	1.55 (1.42, 1.67)	1.48 (1.43, 1.58)	0.11
Categories of 25OHD and PTH			
Low 25OHD (< 20 ng/mL), n (%)	147 (48%)	79 (44%)	0.36
Elevated PTH (> 65 pg/mL), n (%)	27 (9%)	6 (3%)	0.02
25OHD < 20 ng/mL or PTH > 65 pg/mL, n (%)	157 (51%)	79 (44%)	0.11

IQR=interquartile range (25^{th} percentile, 75^{th} percentile); PTH=parathyroid hormone; FGF=fibroblast growth factor; RU=Relative Unit.

[1] Serum concentrations were not measured for 1 participant for calcium (PHIV), for 3 participants for PTH (1 PHIV, 2 PHEU), and for 246 participants for FGF-23 (160 PHIV, 86 PHEU).

[2] p-value by Wilcoxon rank sum test for comparison of medians and by Chi-Square test for comparison of proportions.

Table 3.

Association of cardiac outcomes by 25OHD status among participants from the Adolescent Master Protocol of the Pediatric HIV/AIDS Cohort Study

	Unadjusted Means (SE), z-score				Adjusted Means (SE)[1], z-score			
Cardiac Outcomes	**N**	**Low 25OHD**	**Normal 25OHD**	**P-value**	**N**	**Low 25OHD**	**Normal 25OHD**	**P-value**
Primary Outcome Measures								
Fractional Shortening	483	0.17 (0.06)	0.19 (0.06)	0.79	429	0.20 (0.08)	0.21 (0.06)	0.92
LV Mass	470	0.08 (0.06)	0.37 (0.06)	0.001	400	0.18 (0.07)	0.37 (0.06)	0.05
Contractility	393	0.20 (0.07)	0.26 (0.07)	0.55	339	0.20 (0.08)	0.24 (0.07)	0.72
LV ED Wall Thickness	477	−0.18 (0.06)	−0.12 (0.06)	0.44	406	−0.16 (0.07)	−0.10 (0.06)	0.52
ED Septal Thickness	477	−0.51 (0.06)	−0.45 (0.05)	0.43	406	−0.51 (0.07)	−0.47 (0.06)	0.61
NT-proBNP (pg/mL)	383	1.42 (0.03)	1.51 (0.03)	0.05	334	1.44 (0.04)	1.48 (0.04)	0.42
Secondary Outcome Measures								
LV ED Volume	471	−0.08 (0.07)	0.16 (0.06)	0.01	401	−0.03 (0.07)	0.06 (0.06)	0.34
LV ES Volume	471	−0.17 (0.07)	0.11 (0.06)	0.003	401	−0.09 (0.07)	0.01 (0.06)	0.29
LV Ejection Fraction	472	0.02 (0.06)	0.04 (0.06)	0.83	402	0.03 (0.07)	0.01 (0.06)	0.86
LV ED Dimension	477	−0.36 (0.07)	−0.22 (0.07)	0.13	406	−0.26 (0.08)	−0.25 (0.07)	0.86
M-Mode Thickness-to-Dimension Ratio	478	−0.25 (0.06)	−0.32 (0.05)	0.43	407	−0.30 (0.07)	−0.27 (0.06)	0.73
ES Wall Stress	406	−1.04 (0.08)	−1.22 (0.07)	0.09	366	−1.11 (0.09)	−1.31 (0.08)	0.07

LV=left ventricular; ED=end-diastolic; ES=end-systolic; NT-proBNP= b-type natriuretic peptide; SE=standard error

[1]Association between cardiac outcomes and 25OHD status based on linear regression models adjusted for HIV status, age, sex, race, BMI z-score, and physical activity for all cardiac outcomes, except fractional shortening (adjusted for HIV status, region, and physical activity) and ES wall stress (adjusted for HIV status, age, region, and physical activity).

Table 4.

Association of cardiac outcomes by PTH status among participants from the Adolescent Master Protocol of the Pediatric HIV/AIDS Cohort Study

	Unadjusted Means (SE), z-score				Adjusted Means (SE)[1], z-score			
Cardiac Outcomes	**N**	**Elevated PTH**	**Normal PTH**	**P-value**	**N**	**Elevated PTH**	**Normal PTH**	**P-value**
Primary Outcome Measures								
Fractional Shortening	480	0.38 (0.16)	0.16 (0.04)	0.20	426	0.55 (0.18)	0.18 (0.05)	0.05
LV Mass	467	−0.04 (0.16)	0.25 (0.05)	0.08	397	−0.05 (0.17)	0.31 (0.05)	0.05
Contractility	390	0.26 (0.19)	0.23 (0.05)	0.89	336	0.26 (0.22)	0.22 (0.06)	0.87
LV ED Wall Thickness	474	0.17 (0.15)	−0.17 (0.04)	0.04	403	0.15 (0.18)	−0.13 (0.05)	0.13
ED Septal Thickness	474	−0.32 (0.14)	−0.48 (0.04)	0.27	403	−0.45 (0.16)	−0.48 (0.05)	0.83
Log_{10} NT-proBNP (pg/mL)	380	1.43 (0.09)	1.48 (0.02)	0.63	331	1.47 (0.10)	1.48 (0.03)	0.91
Secondary Outcome Measures								
LV ED Volume	468	0.06 (0.17)	0.04 (0.05)	0.91	398	−0.08 (0.17)	0.02 (0.05)	0.57
LV ES Volume	468	−0.07 (0.18)	−0.01 (0.05)	0.73	398	−0.20 (0.17)	−0.01 (0.05)	0.29
LV Ejection Fraction	469	−0.04 (0.15)	0.03 (0.04)	0.67	399	0.09 (0.18)	0.00 (0.05)	0.64
LV ED Dimension	474	−0.19 (0.18)	−0.30 (0.05)	0.56	403	−0.36 (0.19)	−0.25 (0.05)	0.57
M-Mode Thickness-to-Dimension Ratio	475	−0.03 (0.15)	−0.30 (0.04)	0.077	404	−0.00 (0.17)	−0.29 (0.05)	0.09
ES Wall Stress	403	−1.36 (0.20)	−1.11 (0.05)	0.21	363	−1.50 (0.21)	−1.22 (0.06)	0.19

LV=left ventricular; ED=end-diastolic; ES=end-systolic; NT-proBNP=b-type natriuretic peptide; SE=standard error

[1]Association between cardiac outcomes and 25OHD status based on linear regression models adjusted for HIV status, age, sex, race, BMI z-score, and physical activity for all cardiac outcomes, except fractional shortening (adjusted for HIV status, region, and physical activity) and ES wall stress (adjusted for HIV status, age, region, and physical activity).

Table 5.

Associations of bone mineral metabolism markers with cardiac outcomes among black participants in the Adolescent Master Protocol of the Pediatric HIV/AIDS Cohort Study

	25OHD status							
Cardiac Outcomes	**Unadjusted Means (SE)**				**Adjusted Means (SE)[1]**			
	N	**Low**	**Normal**	**P-value**	**N**	**Low**	**Normal**	**P-value**
Contractility, z-score	269	0.16 (0.08)	0.38 (0.08)	0.05	239	0.19 (0.09)	0.37 (0.09)	0.16
LV Mass, z-score	325	0.03 (0.07)	0.27 (0.08)	0.02	283	0.07 (0.07)	0.21 (0.08)	0.20
LV ED Volume, z-score	326	−0.13 (0.07)	0.10 (0.08)	0.04	284	−0.11 (0.07)	−0.02 (0.08)	0.37
LV ES Volume, z-score	326	−0.19 (0.08)	0.05 (0.08)	0.03	284	−0.15 (0.07)	−0.09 (0.08)	0.54
	Calcium							
	N	**Unadjusted Estimate (SE)**		**P-value**	**N**	**Adjusted Estimate (SE)***		**P-Value**
LV ED Dimension, z-score	331	0.18 (0.10)		0.05	289	0.20 (0.10)		0.04
ES Wall Stress, z-score	279	0.25 (0.11)		0.02	249	0.20 (0.12)		0.08
NT-proBNP (pg/mL)	256	−0.07 (0.05)		0.16	231	−0.16 (0.05)		0.004
	Phosphate							
	N	**Unadjusted Estimate (SE)**		**P-value**	**N**	**Adjusted Estimate (SE)***		**P-Value**
LV Mass, z-score	325	0.20 (0.07)		0.005	283	0.14 (0.08)		0.06
LV ES Volume, z-score	326	0.15 (0.08)		0.05	284	0.03 (0.07)		0.67
NT-proBNP (pg/mL)	257	0.00 (0.04)		0.96	232	−0.08 (0.04)		0.05
	Log_{10} FGF-23							
	N	**Unadjusted Estimate (SE)**		**P-value**	**N**	**Adjusted Estimate (SE)***		**P-Value**
M-Mode Thickness-to-Dimension Ratio, z-score	164	−0.28 (0.30)		0.35	144	−0.66 (0.34)		0.05
ED Septal Thickness, z-score	164	−0.79 (0.30)		0.01	144	−0.77 (0.34)		0.03

LV=left ventricular; ED=end-diastolic; ES=end-systolic; NT-proBNP=b-type natriuretic peptide; SE=standard error

[1] Association between cardiac outcomes and individual bone mineral metabolism markers is adjusted by HIV status, age, sex, BMI z-score, and physical activity for all cardiac outcomes, except Fractional Shortening and ES Wall Stress. The model fitting the association between Fractional Shortening z-score and individual bone mineral metabolism markers is adjusted by HIV status, region, and physical activity, while that between ES Wall Stress and individual bone mineral metabolism markers is adjusted by HIV status, age, region, and physical activity.